Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 5072–5075, April 1998
Developmental Biology

Rescue of *Caenorhabditis elegans* pharyngeal development by a vertebrate heart specification gene

CHRISTINA HAUN*, JONATHAN ALEXANDER†, DIDIER Y. STAINIER†, AND PETER G. OKKEMA*‡

*Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607; and †Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94143

Communicated by Judith Kimble, University of Wisconsin-Madison, Madison, WI, February 17, 1998 (received for review January 6, 1998)

ABSTRACT **Development of pharyngeal muscle in nematodes and cardiac muscle in vertebrates and insects involves the related homeobox genes *ceh-22*, *nkx2.5*, and *tinman*, respectively. To determine whether the nematode and vertebrate genes perform similar functions, we examined activity of the zebrafish *nkx2.5* gene in transgenic *Caenorhabditis elegans*. Here, we report that ectopic expression of *nkx2.5* in *C. elegans* body wall muscle can directly activate expression of both the endogenous *myo-2* gene, a *ceh-22* target normally expressed only in pharyngeal muscle, and a synthetic reporter construct controlled by a multimerized CEH-22 binding site. *nkx2.5* also efficiently rescues a *ceh-22* mutant when expressed in pharyngeal muscle. Together, these results indicate that *nkx2.5* and *ceh-22* provide a single conserved molecular function. Further, they suggest that an evolutionarily conserved mechanism underlies heart development in vertebrates and insects and pharyngeal development in nematodes.**

Heart development in animals as distantly related as insects and vertebrates involves related NK-2 family homeobox genes (reviewed in ref. 1). In *Drosophila*, the *tinman* homeobox gene is expressed in cardiac precursors, and *tinman* mutants completely lack a heart (2–4). Likewise in vertebrates, the *nkx2.5* homeobox gene is expressed in myocardial precursors (5–9), and mouse *Nkx2–5* mutants exhibit defects in cardiac morphogenesis and gene expression (10). This remarkable molecular conservation suggests a common mechanism controls heart development in a wide variety of species.

Nematodes have no heart or defined circulatory system; however, existing evidence suggests the nematode pharynx, a rhythmically contracting organ involved in feeding, shares functional and molecular similarities with the heart in other species. Pharyngeal muscle function, like that of vertebrate cardiac muscle, does not require nervous system input (11). Likewise at the molecular level, pharyngeal muscle development does not involve the MyoD family of myogenic regulatory factors (12, 13). Instead, pharyngeal muscle development requires the *tinman/nkx2.5*-related homeobox gene *ceh-22*. *ceh-22* is expressed exclusively in pharyngeal muscle, where it binds the enhancer of the pharyngeal muscle-specific *myo-2* gene, and a *ceh-22* mutant displays defects in pharyngeal morphology and function (13, 14).

Is there a relationship between heart development in insects and vertebrates and pharyngeal development in nematodes? To address this question, we have examined the ability of the zebrafish *nkx2.5* gene (8, 9) to substitute for *ceh-22* in transgenic *C. elegans*. We have found that, like *ceh-22* (15), *nkx2.5* can activate expression of *myo-2* when expressed ectopically in body wall muscle and that *nkx2.5* can rescue a *ceh-22* mutant when expressed in pharyngeal muscle. These results indicate that *ceh-22* and *nkx2.5* share a common function and suggest that development of the nematode pharynx and the vertebrate heart may involve a conserved regulatory mechanism.

MATERIALS AND METHODS

Plasmids and *C. elegans* Transformation Techniques. To produce *unc-54::nkx2.5*, an *nkx2.5* cDNA, PCR amplified from adult zebrafish heart using primers derived from the published sequence (8, 9) was inserted downstream of the *unc-54* promoter in pPD30.38 (16) using an *Nco*I site within the *nkx2.5* 5′-untranslated region. To produce *ceh-22::nkx2.5* (designated plasmid pOK102.01) or *ceh-22::ceh-22* (designated plasmid pOK102.05), cDNAs were inserted downstream of the *ceh-22* promoter from pOK29.02 (14).

Two transgenic lines containing *unc-54::nkx2.5* were isolated by microinjection into wild-type N2 *C. elegans*, using the cotransformation marker pRF4 (17). To produce *ceh-22(cc8266)* mutant lines bearing either *ceh-22::nkx2.5* or *ceh-22::ceh-22* expression vectors, the balanced heterozygous strain OK 0060 [*ceh-22(cc8266)/unc-42(e270) sma-1(e30)*] was transformed with each construct using the pRF4 marker; two transformed homozygous *ceh-22(cc8266)* lines segregating from each of these initial heterozygous transformants were identified and characterized.

Antibody Staining and Expression of *lacZ* Reporters. Expression of MYO-2 protein was examined by staining with the monoclonal antibody 9.2.1 [kindly provided by D. M. Miller (18)] following whole mount fixation of mixed-stage populations of nematodes (19, 20). Primary antibodies were detected by immunofluoresence microscopy using Texas red-conjugated goat-anti mouse IgG (Jackson ImmunoResearch) secondary antibodies. To assay function of *lacZ* reporters, F_1 expression assays were carried out as previously described (21); plasmids pOK21.43 (multimerized *B* sub-element reporter) and pOK29.02 (*ceh-22::lacZ*) were microinjected at 100 μg/ml into the germ line of adult hermaphrodites and F_1 progeny stained for β-galactosidase activity as late larvae or adults.

RESULTS

Zebrafish *nkx2.5* Can Activate *myo-2* Expression When Expressed in *C. elegans* Body Wall Muscle. In wild-type *C. elegans*, *ceh-22* is expressed exclusively in pharyngeal muscle where it activates expression of the pharyngeal muscle-specific myosin heavy chain gene *myo-2* (14). Ectopic expression of *ceh-22* in body wall muscle can activate expression of *myo-2* (15); because *myo-2* is normally never expressed in body wall muscle, this ectopic expression assay provides a sensitive test for *ceh-22* function. To determine whether zebrafish *nkx2.5* can function similarly to *ceh-22*, we expressed *nkx2.5* in *C. elegans* body wall muscle and examined expression of the endogenous

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

‡To whom reprint requests should be addressed at: Department of Biological Sciences (MC 567), University of Illinois at Chicago, 900 S. Ashland Ave., Rm. 4150, Chicago, IL 60607. e-mail: okkema@uic.edu.

myo-2 gene by antibody staining. Two transgenic lines expressing an *nkx2.5* cDNA under the control of the *unc-54* body wall muscle-specific promoter were generated. In both lines, we detected *myo-2* expression in the body wall muscles (Fig. 1 *A* and *B*). These results show that *nkx2.5* can function like *ceh-22* to induce *myo-2* expression.

We next asked whether Nkx2.5 directly interacts with the same sequences recognized by CEH-22 by examining expression of a reporter gene under the control of multimerized CEH-22 binding sites. CEH-22 binds a region within the *myo-2* enhancer termed the *B* sub-element (14). In wild-type animals, a *lacZ* reporter under control of a synthetic enhancer consisting of four copies of a 28-bp *B* sub-element oligonucleotide is expressed specifically in pharyngeal muscle; only occasional expression is observed outside the pharynx (Table 1; ref. 14). In a transgenic strain bearing the *unc-54*::*nkx2.5* expression construct, we found a significant increase in the number of animals expressing β-galactosidase in body wall muscle (from 2.5 to 16.5%) (Table 1; Fig. 1*C*). To rule out the possibility that Nkx2.5 was indirectly enhancing expression of *myo-2* or the *B* sub-element reporter by activating ectopic expression of the *ceh-22* gene, we examined expression of a *ceh-22*::*lacZ* fusion in animals bearing the *unc-54*::*nkx2.5* transgene. Expression of β-galactosidase was limited to pharyngeal muscle (Table 1), a pattern identical to that observed in wild-type animals (14). Thus, these data together indicate that Nkx2.5, like CEH-22, activates transcription by interacting directly with the *B* sub-element of the *myo-2* enhancer.

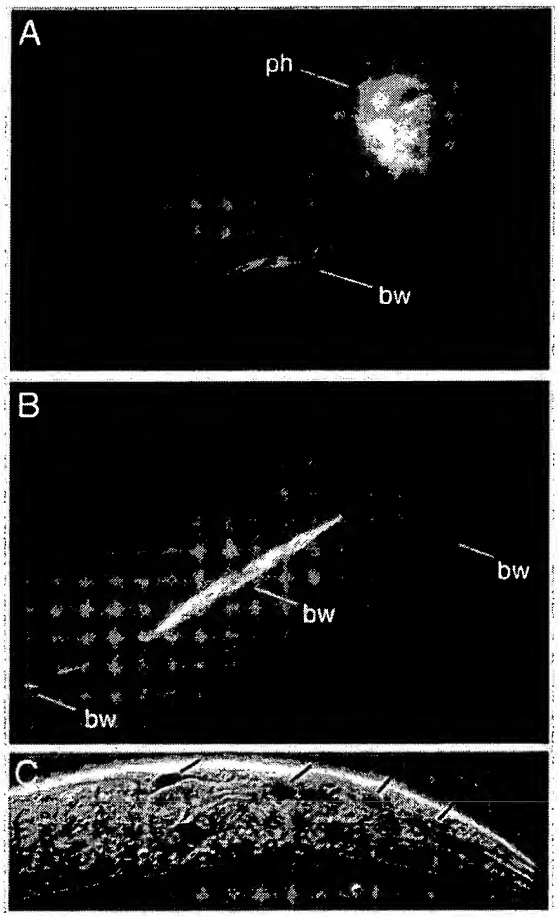


FIG. 1. Expression of zebrafish *nkx2.5* in *C. elegans* body wall muscle activates expression of pharyngeal muscle-specific genes. (*A* and *B*) Adult animals bearing the *unc-54*::*nkx2.5* expression vector stained with the anti-MYO-2 antibody, 9.2.1. MYO-2 protein accumulates in pharyngeal muscles (ph) and body wall muscles (bw). Animals ectopically expressing either *ceh-22* or *nkx2.5* generally express MYO-2 in only a few (approximately 1–5) body wall muscle cells (15); the numbers of MYO-2 positive body wall muscles are similar in animals expressing either *ceh-22* or *nkx2.5*. (C) An adult animal bearing the *unc-54*::*nkx2.5* transgene and expressing a *B* sub-element::*lacZ* reporter in body wall muscles stained with X-Gal. β-Galactosidase staining accumulates in body wall muscle nuclei (marked by black bars).

Table 1. Expression of *lacZ* reporters in body wall muscle

Construct	Percent F_1 transformants expressing β-galactosidase in body wall muscle*	
	Wild type (N2)	OK0027†
B sub-element::*lacZ*	2.5% (n = 118)	16.5% (n = 151)
ceh-22::*lacZ*	ND	0% (n = 69)

**lacZ* reporters were injected into the germ line of adult hermaphrodites, and the entire F_1 brood was stained to detect β-galactosidase expression (21). Using this "F_1 expression assay," both reporters are strongly expressed in pharyngeal muscle (14). The total number of F_1 transformants (n), recognized by pharyngeal expression of β-galactosidase, was counted, and they were then examined for ectopic β-galactosidase expression in body wall muscle.

†The *unc-54*::*nkx2.5* fusion in strain OK 0027 is maintained as an extrachromosomal array that is segregated to 55% of F_1 progeny; therefore, approximately 30% of the F_1 transformants bearing the *unc-54*::*nkx2.5* fusion express *B* sub-element::*lacZ* in body wall muscle.

***nkx2.5* Can Substitute for *ceh-22* During Normal Pharyngeal Development.** In addition to its role in *myo-2* activation, CEH-22 likely regulates other genes required for pharyngeal development. Indeed, a *ceh-22* mutant exhibits profound contractile and morphological defects in the pharynx, despite expressing *myo-2* nearly as well as wild type (15). To examine the extent to which Nkx2.5 and CEH-22 are functionally equivalent, we asked if expression of *nkx2.5* in pharyngeal muscle could rescue a *ceh-22* mutant. The loss-of-function mutation *ceh-22(cc8266)* causes pharyngeal muscle defects that interfere with feeding, resulting in a lethal/slow growth phenotype (15). Approximately one-quarter of *ceh-22(cc8266)* mutants arrest shortly after hatching, whereas the remaining mutants grow slowly to adulthood. These mutant adults exhibit characteristic morphological and contractile defects in the pharynx and have a starved appearance typical of feeding defective mutants. We placed the *nkx2.5* cDNA under control of the *ceh-22* promoter and introduced this fusion (designated *ceh-22*::*nkx2.5*) into *ceh-22(cc8266)* mutants. For comparison, we also introduced a similar fusion containing the *ceh-22* cDNA under the control of the *ceh-22* promoter (designated *ceh-22*::*ceh-22*) into *ceh-22(cc8266)* mutants.

We tested whether expression of *nkx2.5* or *ceh-22* could rescue the larval arrest/slow growth phenotype by picking synchronized embryos and counting the number of animals reaching adulthood after 4 days at 20°C (Table 2). Ninety-eight percent of the wild-type embryos reached adulthood under these conditions. In comparison, only 36% of the untransformed *ceh-22(cc8266)* mutants reached adult stage, and these adults had a thin, pale appearance indicative of poor feeding (22). For *ceh-22(cc8266)* mutants transformed with *ceh-22*::*nkx2.5* or *ceh-22*::*ceh-22*, 68% and 77% of the animals reached adulthood within 4 days, respectively, and all of these transformed adults had a well fed appearance. The animals that did not reach adulthood likely did not contain the transforming DNA, which in *C. elegans* is maintained as a semi-stable extrachromosomal array (17); however, we cannot rule out the possibility that neither *nkx2.5* nor *ceh-22* completely rescued the mutant phenotype. Nonetheless, the ability of the *ceh-22*::*nkx2.5* transgene to rescue *ceh-22(cc8266)* growth defects nearly as well as *ceh-22*::*ceh-22* was quite striking.

Table 2. *nkx2.5* and *ceh-22* rescue the growth defects in *ceh-22(cc8266)*

Genotype	Percent animals reaching adult after 4 days at 20°C*	*n*
Wild type (N2)	98	305
ceh-22(cc8266)	36	230
ceh-22(cc8266); cuEx(ceh-22::nkx2.5 cDNA)	68	104
ceh-22(cc8266); cuEx(ceh-22::ceh-22 cDNA)	77	203

*Embryos segregating from the indicated strains were picked to fresh plates and incubated at 20°C. After 4 days, the number of animals reaching adulthood was determined. Neither *ceh-22::ceh-22* nor *ceh-22::nkx2.5* rescued a partially penetrant sterile phenotype exhibited by *ceh-22(cc8266)* (ref. 15; data not shown). This phenotype is rescued by transformation with a *ceh-22* genomic DNA fragment; thus, we believe the *ceh-22::ceh-22* and *ceh-22::nkx2.5* may be missing sequences necessary for rescue of sterility.

We also examined the pharyngeal morphology of representative adult *ceh-22(cc8266)* mutants and *ceh-22(cc8266)* animals bearing either the *ceh-22::nkx2.5* or *ceh-22::ceh-22* fusion by Nomarski microscopy (Fig. 2). Nine of nine untransformed *ceh-22(cc8266)* mutants displayed an abnormal pharyngeal morphology (Fig. 2*B*). The pharynx appeared thicker than wild type, particularly in the region where the isthmus joins the terminal bulb; in addition, the grinder, a cuticular structure located in the lumen of the pharynx, appeared in an abnormal orientation and did not invert completely during pharyngeal pumping. In contrast, nearly all of the *ceh-22(cc8266)* animals bearing *ceh-22::nkx2.5* or *ceh-22::ceh-22* displayed a normal pharyngeal morphology (Fig. 2 *C* and *D*); only 1/17 and 1/19 of these adults, respectively, appeared abnormal, whereas the pharyngeal contractions and inversion of the grinder appeared normal in all transgenic animals.

DISCUSSION

Our results demonstrate that zebrafish *nkx2.5* and *ceh-22* are functionally interchangeable in *C. elegans*. Nkx2.5 can bind sites within the chromosome normally targeted by CEH-22 and

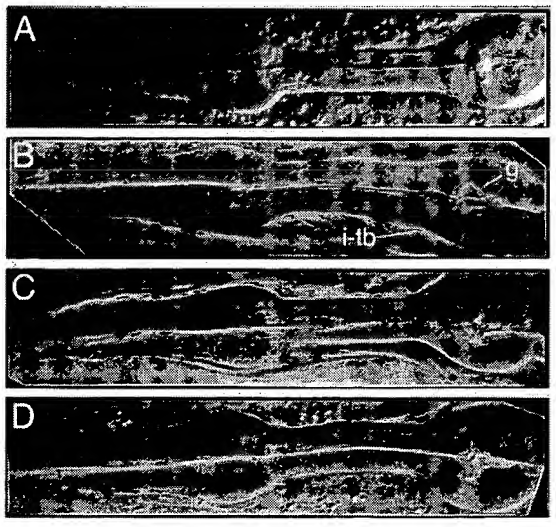


FIG. 2. Expression of *nkx2.5* or *ceh-22* in pharyngeal muscle rescues a *ceh-22* mutant. Nomarski micrographs of the adult pharynx in wild type (*A*), a *ceh-22(cc8266)* mutant (*B*), and a *ceh-22(cc8266)* mutant rescued with either the *ceh-22::nkx2.5* (*C*) or the *ceh-22::ceh-22* (*D*) expression vectors. The poorly formed grinder (g) and thickened junction of the pharyngeal isthmus and terminal bulb (i-tb) characteristic of *ceh-22(cc8266)* are marked in *B*.

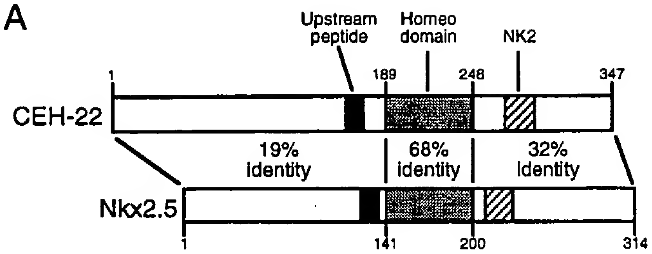
interact productively and specifically with the *C. elegans* transcriptional machinery. Mutational analysis of the *B* subelement of *myo-2* indicates that CEH-22 interacts with additional *C. elegans* factors to regulate gene expression (14). Whatever the nature of the interactions with these other as yet unidentified proteins, Nkx2.5 seems capable of reproducing them. Amino acid sequence identity between Nkx2.5 and CEH-22 is greatest within the homeodomain (68% identity), although two short regions of similarity are also found in conserved positions upstream and downstream of the homeodomain (Fig. 3). The region of conservation downstream of the homeodomain is part of an NK2-specific domain like that found in Nkx2.5 (1, 8, 9); however, deletion of this divergent NK2 domain from CEH-22 has no detectable effect on its ability to activate *myo-2* when expressed in body wall muscle (R. Reardon and P.G.O., unpublished work). It therefore seems likely that the homeodomain and perhaps the conserved upstream peptide are the primary determinants mediating function of CEH-22 and Nkx2.5, although other regions of low primary sequence identity but high functional homology may also contribute to activity.

Similar experiments examining function of murine *Nkx2–5* in *Drosophila* indicate that derivatives of *Nkx2–5* can also partially rescue the *tinman* mutant, suggesting a conservation of function also exists between *tinman* and *Nkx2–5* (R. Bodmer, personal communication; G. Ranganayakulu, R. Harvey, and E. Olson, personal communication). Together these results and ours suggest that a key step in the development of cardiac muscle in vertebrates and insects and pharyngeal muscle in nematodes is controlled by a common factor. Expression of *tinman* and *nkx2.5* in the hearts of *Drosophila* (23, 24) and the chicken, respectively (25), involve upstream signals mediated by transforming growth factor-β superfamily and *wingless* signaling pathways; it will be important to determine whether similar upstream signals regulate *ceh-22* expression in the *C. elegans* pharynx to understand the extent to which the pathways specifying heart and pharyngeal development are conserved.

Although *ceh-22, nkx2.5*, and *tinman* seem to share common function, it is not clear that they are orthologs. These genes are members of the larger family of NK2 homeobox genes and other family members function in a variety of tissues (1). Indeed the CEH-22 homeodomain shares highest sequence identity with mouse *Nkx2–2* and *Drosophila vnd* (14), which are primarily expressed in the developing nervous system (26, 27). It will be interesting to determine whether these or other NK2 family members will also function like *nkx2.5* to activate *myo-2*. It is noteworthy that another *C. elegans* NK2 homeobox gene, *ceh-24*, is expressed outside the pharynx in some neurons and the vulval muscles (28), suggesting certain NK2 family members may be incapable of activating *myo-2*.

The nematode pharynx, vertebrate heart, and insect heart are all muscular tubes that pump liquids, although the specific functions of these organs are very different. What is the significance of the fact that these different organs utilize a common myogenic mechanism? One interesting observation is that in all vertebrates thus far examined, *nkx2.5* (or the related genes *nkx2.3* and *nkx2.7*) is expressed not only in the anterior lateral plate mesoderm, which will give rise to the heart, but also in the anterior endoderm, which will give rise to parts of the anterior digestive tract (5–9). Likewise, *Drosophila tinman* is transiently expressed in an anterior region of the embryo surrounding the stomodeum (2), which gives rise to the mouth, pharynx, and esophagus (29). Perhaps the ancestral function of *ceh-22/tinman/nkx2.5* was to demarcate a "module" (as defined in ref. 30) that gave rise to an anteriorly located contractile tube used for feeding, circulation, or both. The mesodermal component of this field may have been co-opted during evolution to form the very distinct structures found in modern phyla.

A



B

Homeodomain

```
CEH-22  (189)KRKRRVLFTKAQTYELERRFRSQKYLSAPEREALAMQIRLTPTQVKIWFQNHRYKTKKSH(248)
              .:: ::::..::.:::.:::..:::::::::. ::  ..:: :::::::::.::: :...
Nkx2.5  (141)RRKPRVLFSQAQVYELQRRFKQQKYLSAPERDHLANVLKLTSTQVKIWFQNRRYKCKRQR(200)
```

Upstream peptide

```
CEH-22  (163)IKLEDED(169)
              .:::.: :
Nkx2.5  (128)LKLDDAD(134)
```

NK2 domain

```
CEH-22  (276)TRAMPIPMLVRDSSAR(291)
               :  .  .:.:::::..
Nkx2.5  (213)PRRISVPVLVRDGKPC(228)

Cons         PRRVAVPVLVRDGKPC
```

FIG. 3. Regions of sequence identity between CEH-22 and Nkx2.5. (*A*) Schematic diagram of CEH-22 and Nkx2.5 indicating the positions of conserved sequences (numbered according to the predicted protein sequence). The overall levels of amino acid sequence identity are reported for the homeodomains and for the portions of the proteins outside the homeodomains. (*B*) Sequence alignments between conserved regions of CEH-22 and Nkx2.5. Amino acid identities are indicated by a colon (:) and similarities by a period (.); numbering indicates the location of these sequences within the primary amino acid sequence. A portion of the NK2-specific domain consensus reported in ref. 1 is shown with residues present in both CEH-22 and Nkx2.5 underlined.

We are grateful to Andrew Fire for encouragement and critical reading of this manuscript. This work was funded by a grant from National Institutes of Health (to P.G.O.) and grants from National Institutes of Health, American Heart Association, and the March of Dimes and Packard Foundations (to D.Y.R.S.). J.A. is currently an American Heart Association predoctoral fellow and a trainee in the Medical Scientist Training Program at University of California, San Francisco. Some nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health National Center for Research Resources.

1. Harvey, R. (1996) *Dev. Biol.* **178,** 203–216.
2. Bodmer, R., Jan, L. Y. & Jan, Y. N (1990) *Development* **110,** 661–669.
3. Bodmer, R. (1993) *Development* **118,** 719–729.
4. Azpiazu, N. & Frasch, M. (1993) *Genes Dev.* **7,** 1325–1340.
5. Lints, T. J., Parsons, L. M., Hartley, L., Lyons, I. & Harvey, R. P. (1993) *Development* **119,** 419–431.
6. Tonissen, K. F., Drysdale, T. A., Lints, T. J., Harvey, R. P. & Krieg, P. A. (1994) *Dev. Biol.* **162,** 325–328.
7. Schultheiss, T., Xydas, S. & Lassar, A. B. (1995) *Development* **121,** 4203–4214.
8. Lee, K., Xu, Q. & Breitbart, R. E. (1996) *Dev. Biol.* **180,** 722–731.
9. Chen, J.-N. & Fishman, M. C. (1996) *Development* **122,** 3809–3816.
10. Lyons, I., Parsons, L. M., Hartley, L., Li, R., Andrews, J. E., Robb, R. & Harvey, R. P. (1995) *Genes Dev.* **9,** 1654–1666.
11. Avery, L. & Horvitz, H. R. (1989) *Neuron* **3,** 473–485.
12. Krause, M., Fire, A., White Harrison, S., Priess, J. & Weintraub, H. (1990) *Cell* **63,** 907–919.
13. Chen, L., Krause, M., Sepanski, M. & Fire, A. (1994) *Development* **120,** 1631–1641.
14. Okkema, P. G. & Fire, A. (1994) *Development* **120,** 2175–2186.
15. Okkema, P. G., Ha, E., Haun, C., Chen, W. & Fire, A. (1997) *Development* **124,** 3965–3973.
16. Mello, C. C. & Fire, A. (1995) in *Methods in Cell Biology: C. elegans*, eds. Shakes, D. & Epstein, H. (Academic, San Diego), pp. 452–482.
17. Mello C. C., Kramer, J. M., Stinchcomb, D. & Ambros, V. (1991) *EMBO J.* **10,** 3959–3970.
18. Miller, D. M., Stockdale, F. E. & Karn, J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2305–2309.
19. Miller, D. M. & Shakes, D. C. in *Methods in Cell Biology: C. elegans*, eds. Shakes, D. & Epstein, H. (Academic, San Diego), pp. 365–394.
20. Finney, M. & Ruvkun, G. B. (1990) *Cell* **63,** 895–905.
21. Okkema, P. G., Harrison, S. W., Plunger, V., Aryana, A. & Fire, A. (1993) *Genetics* **135,** 385–404.
22. Avery, L. (1993) *Genetics* **133,** 897–917.
23. Frasch, M. (1995) *Nature* **374,** 464–467.
24. Park, M., Wu, X., Golden, K., Axelrod, J. D. & Bodmer, R. (1996) *Dev. Biol.* **177,** 104–116.
25. Schultheiss, T. M., Burch, J. B. & Lassar, A. B. (1997) *Genes Dev.* **11,** 451–462.
26. Jimenez, F., Martin-Morris, L. E., Velasco, L., Chu, H., Sierra, J., Rosen, D. R. & White K. (1995) *EMBO J.* **14,** 3487–3495.
27. Price, M., Lazzaro, D., Pohl, T., Mattei, M. G., Ruther, U., Olivo, J. C., Duboule, D. & Di Lauro, R. (1992) *Neuron* **8,** 241–255.
28. Harfe, B. & Fire, A. (1998) *Development* **125,** 421–429.
29. Fullilove, S. L. & Jacobson, A. G. (1978) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Wright, T. R. F. (Academic, New York), Vol. 2c, pp. 105–227.
30. Raff R. A. (1996) *The Shape of Life: Genes, Development, and the Evolution of Animal Form* (University of Chicago Press, Chicago).

EXHIBIT 2

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 14940–14944, December 1996
Neurobiology

Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*

(Alzheimer disease/*sel-12*/genetics/transgenic nematode/expression)

DIANE LEVITAN*†, TIMOTHY G. DOYLE‡, DENISE BROUSSEAU*, MICHAEL K. LEE§, GOPAL THINAKARAN§, HILDA H. SLUNT§, SANGRAM S. SISODIA§¶, AND IVA GREENWALD*†‖

*Howard Hughes Medical Institute, †Department of Biochemistry and Molecular Biophysics, and ‡Integrated Program in Biochemistry and Molecular Biophysics, College of Physicans and Surgeons, Columbia University, New York, NY 10032; and Departments of §Pathology and ¶Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Communicated by Thomas W. Cline, University of California, Berkeley, CA, October 1, 1996 (received for review September 26, 1996)

ABSTRACT **We provide evidence that normal human presenilins can substitute for *Caenorhabditis elegans* SEL-12 protein in functional assays *in vivo*. In addition, six familial Alzheimer disease-linked mutant human presenilins were tested and found to have reduced ability to rescue the *sel-12* mutant phenotype, suggesting that they have lower than normal presenilin activity. A human presenilin 1 deletion variant that fails to be proteolytically processed and a mutant SEL-12 protein that lacks the C terminus display considerable activity in this assay, suggesting that neither presenilin proteolysis nor the C terminus is absolutely required for normal presenilin function. We also show that *sel-12* is expressed in most neural and nonneural cell types in all developmental stages. The reduced activity of mutant presenilins and as yet unknown gain-of-function properties may be a contributing factor in the development of Alzheimer disease.**

Genetic linkage studies have identified a number of genetic loci associated with familial Alzheimer disease (1). Mutations in two genes, encoding the presenilins PS1 and PS2, are dominant and fully penetrant (1–5). PS1 and PS2 are related multipass transmembrane proteins that are about 67% identical in amino acid sequence. The presenilins are ubiquitously expressed (4, 5) and found in conjunction with intracellular membranes (6).

The normal function of presenilins and the mechanism by which mutant presenilins cause Alzheimer disease are not yet known. An understanding of the normal function of presenilins and of the nature of the dominant mutations is crucial to elucidating the role of mutant presenilins in Alzheimer disease. The fact that more than 30 dominant, fully penetrant mutations in PS1 and PS2 are all missense mutations has suggested that Alzheimer disease is associated with a gain-of-function activity of mutant proteins, although it remains possible that they partially lower activity of a dose-sensitive gene. Classical studies have indicated that gain-of-function mutations in principle fall into one of three classes: hypermorphic mutations, which elevate gene activity; antimorphic mutations, which reduce wild-type gene activity in trans (this category includes dominant-negative mutations); and neomorphic mutations, which create a novel activity (7). Thus, the mechanism by which hypermorphic and antimorphic mutations exert their effects is by altering the level of normal gene activity. Studies of neomorphic missense mutations suggest that they cause relatively subtle changes, so that at the biochemical level, the novel activity resulting from a neomorphic mutation still relates to the normal mechanism of gene function. For example, neomorphic mutations in the *Drosophila* abnormal wing discs (*awd*) gene appear to alter the substrate specificity of nucleoside diphosphate kinase and to reduce activity for its normal substrate (8), and mutations that cause familial amyotropic lateral sclerosis affect different activities of the normal protein, increasing the level of peroxidase activity (9), while in some cases reducing superoxide dismutase activity (10). It is notable that in these cases the neomorphic mutations have both gain-of-function and loss-of-function characteristics.

Genetic studies in simple organisms offer a powerful approach to understanding the normal role of presenilins. A *Caenorhabditis elegans* gene, *sel-12*, encodes a protein that displays about 50% amino acid sequence identity to PS1 and PS2 (11). *sel-12* was identified by reverting a phenotype caused by constitutive activation of LIN-12, a member of the LIN-12/Notch family of receptors (*sel* is suppressor/enhancer of *lin-12*). Genetic analysis established that reducing or eliminating *sel-12* activity reduces the activity of *lin-12* and causes an egg-laying defective (Egl) phenotype. The Egl phenotype may be a direct consequence of reducing *lin-12* activity (12) or an independent effect of reducing *sel-12* activity. In this paper, we provide evidence that SEL-12 and the presenilins are functional homologs and that studies in *C. elegans* will be directly applicable to issues of presenilin structure and function in humans.

MATERIALS AND METHODS

General Methods and Mutations Used. Methods for handling and culturing *C. elegans* have been described (13). The wild-type parent for all strains used was *C. elegans* var. Bristol strain N2 (13). *sel-12(ar131)* is described in ref. 11. All strains containing pLEX-based plasmids (see below) contained the *smg-1(r861)* and *unc-54(r293)* mutations (14). *smg-1* mutations stabilize mRNAs with long 3′ untranslated regions (15), and *unc-54(r293)* is suppressed by *smg-1(r861)* (14).

pLEX-Based Constructs. The pLEX vector has been described (16). It contains a 15.1-kb genomic region encompassing the *lin-12* gene, in which the normal translational start ATG was destroyed and replaced with a *Not*I site. cDNAs containing stop codons but lacking polyadenylylation signals are inserted into the *Not*I site and are efficiently expressed in a *smg-1* background. The following cDNAs were inserted into pLEX for this study.

sel-12. The *sel-12* cDNA is described in ref. 11 and, as described below, results in efficient rescue of a *sel-12* mutant. We note that the *C. elegans* genome project has sequenced through the *sel-12* region (*C. elegans* Sequencing Consortium,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: Egl, egg-laying defective.

‖To whom reprint requests should be addressed at: Howard Hughes Medical Institute and Department of Biochemistry and Molecular Biophysics, 701 West 168th Street, Room 720A, College of Physicians and Surgeons, Columbia University, New York, NY 10032. e-mail: greenwald@cuccfa.ccc.columbia.edu.

personal communication). By comparing the genomic sequence with that of the available *sel-12* cDNA, we discovered that the cDNA has a frameshift mutation, beginning at codon 413, probably introduced by reverse transcription. This frameshift results in the substitution of 31 amino acids C terminal to the frameshift mutation by 49 amino acids. The 31-amino acid sequence deleted is 42% identical between SEL-12 and PS1 or PS2.

PS1. Full-length human PS1 cDNA and cDNA encoding the PS1 A246E substitution were generated by reverse transcription-coupled PCR of cytoplasmic RNA isolated from skin fibroblasts of a patient harboring the A246E mutation (National Institute of Aging Cell Repository AG06848B) using a sense primer, hAD3-ATG-Kpn (GGGGTACCATGACAGAGTTACCTGCAC), and antisense primer, hAD3-R-3'UTR (CCGGGATCCATGGGATTCTAACCGC). PCR products were digested with *Asp*718 and *Bam*HI and ≈1.4-kB hPS1 cDNAs were gel-purified and ligated to Bluescript KS+ vector (Stratagene) previously digested with *Asp*718 and *Bam*HI, to generate phPS1 and phPS1A246E. The cDNAs were sequenced in their entirety using a Sequenase kit (United States Biochemical).

To generate human PS1 cDNA encoding the M146L, H163R, L286V, or C410Y substitutions (5), we used a four-way PCR strategy with two primer pairs and full-length PS1 cDNA as template. The inserts and junctions were sequenced using Sequenase.

For M146L, primer pairs were hAD3-M146LF (GTCATTGTTGTCCTGACTATCCTCCTG)/hAD3-R284 (GAGGAGTAAATGAGAGCTGG) and hAD3-M146LR (CAGGAGGATAGTCAGGACAACAATGAC)/hAD3-237F (CAGGTGGTGGAGCAAGATG). PCR products from each reaction were gel-purified, combined, and subject to a second round of PCR with primers hAD3-237F and hAD3-R284. The resulting product was digested with *Kas*I and *Pfl*MI and an ≈300-bp gel-purified fragment was ligated to *Kas*I/*Pfl*MI-digested phPS1 to generate phPS1M146L.

For H163R, primer pairs were hAD3-H163RF (CTAGGTCATCCGTGCCTGGC)/hAD3-R284 and hAD3-H163RR (GCCAGGCACGGATGACCTAG)/hAD3-237F. PCR products from each reaction were gel-purified, combined, and subject to a second round of PCR with primers hAD3-237F and hAD3-R284. The resulting products were digested with *Kas*I and *Pfl*MI and a gel-purified ≈300-bp fragment was ligated to *Kas*I/*Pfl*MI-digested phPS1 to generate phPS1H163R.

For L286V, primer pairs were hAD3-L286VF (CGCTTTTTCCAGCTGTCATTTACTCC)/hAD3-RL-GST (CCGGAATTCTCAGGTTGTGTTCCAGTC) and hAD3-L286VR (GGAGTAAATGACAGCTGGAAAAAGCG)/hAD3-F146 (GGATCCATTGTTGTCATGACTATC). PCR products from each reaction were gel-purified, combined, and subject to a second round of PCR with primers hAD3-F146 and hAD3-RL-GST. The resulting products were digested with *Pfl*MI and *Bbs*I and a gel-purified ≈480-bp fragment was ligated to *Pfl*MI/*Bbs*I-digested phPS1 to generate phPS1L286V.

For C410Y, primer pairs were hAD3-C410YF (CAACCATAGCCTATTTCGTAGCC)/LRT7 (GCCAGTGAATTGTAATACGACTCACTATAGGGC) and hAD3-C410YR (GGCTACGAAATAGGCTATGGTTG)/hAD3-243S (CCGGAATTCTGAATGGACTGCGTG). PCR products from each reaction were gel-purified, combined, and subject to a second round of PCR with primers hAD3-243S and LRT7. The resulting products were digested with *Bbs*I and *Bam*HI and an ≈300-bp fragment was gel-purified and ligated to *Bbs*I/*Bam*HI-digested phPS1 to generate phPS1C410Y.

The strategy for generating cDNA encoding hPS1 lacking exon 9 (amino acids 290–319) was as described (17).

PS2. Full-length cDNA encoding human PS2 was generated by reverse transcription-coupled PCR of total human brain RNA using a sense primer, huAD4-ATGF (CCGGTACCAAGTGTTCGTGGTGCTTCC), and antisense primer, hAD4-stopR (CCGTCTAGACCTCAGATGTAGAGCTGATG). PCR products were digested with *Asp*718 and *Xba*I and ≈1.4-kB hPS2 cDNA were gel-isolated and ligated to a vector fragment from expression plasmid pCB6 (17) previously digested with *Asp*718 and *Xba*I to generate phPS2. The insert was sequenced in its entirety using a Sequenase kit.

Transgenic Lines and Rescue Assays. Transgenic lines were established by microinjection of plasmid mixtures into the hermaphrodite germ line to create extrachromosomal arrays (18). By accepted convention, Ex is used to represent extrachromosomal arrays, and Is to represent integrated arrays (which can be generated from extrachromosomal arrays; see below).

pLEX and derivatives were injected at 20 μg/ml, 2 μg/ml, or other concentrations (data not shown) into recipient strains of genotype *smg-1(r861) unc-54(r293)*; *sel-12(ar131)* or *smg-1(r861) unc-54(r293)*. pRF4, a plasmid containing the cloned dominant *rol-6(su1006)* gene (18) was used as a cotransformation marker and coinjected at a concentration of 100 μg/ml. F_1 Roller progeny were picked, and F_2 Roller progeny were used to establish lines.

To assess rescue of *sel-12(ar131)*, approximately 40 L4 Rol progeny from at least three lines generated in a *smg-1(r861) unc-54(r293)*; *sel-12(ar131)* background were picked individually and scored daily for the ability to lay eggs. We note that rescue assays were performed using *sel-12(ar131)*, a strong partial loss-of-function allele of *sel-12*, because the strongest existing *sel-12* mutation, *sel-12(ar171)*, is somewhat suppressed by *smg-1* (data not shown). The *sel-12(ar131)* egg-laying defect is of variable penetrance (see Fig. 1) and expressivity. About 7% of *smg-1 unc-54; sel-12(ar131)* hermaphrodites have normal egg-laying, while the remainder bloat with retained eggs; some of these bloated hermaphrodites never lay eggs, whereas others lay eggs. However, the proportion of hermaphrodites that lay eggs normally appears to be reduced by the pLEX vector and/or the *rol-6* cotransformation marker (see Fig. 1). We scored hermaphrodites as "Egl$^+$" only if they displayed robust egg-laying characteristic of wild-type hermaphrodites for 2 days as adults, since pLEX-containing control hermaphrodites very rarely lay eggs after 2 days. However, we note that this criterion appears to underestimate rescuing activity, since many hermaphrodites containing human wild-type and mutant presenilins displayed improved egg-laying for 1 day relative to pLEX-containing controls (data not shown) but no longer laid eggs by the second day. The pLEX vector causes a low level of sterility, and sterile hermaphrodites were not scored.

Transgenic Lines and β-Galactosidase Staining. pIB1Z17 [*sel-12::lacZ*] was made as follows: A unique *Bam*HI site was inserted using the PCR at the second amino acid of a *sel-12* rescuing genomic fragment containing 2.8 kb of 5′ flanking region. A *lacZ* gene encoding a β-galactosidase protein containing a nuclear localization signal was excised from plasmid pPD16.43 (19) and inserted in frame into the *Bam*HI site to generate the plasmid pIB1Z17. The predicted transcript contains an abnormally long 3′ untranslated region, consisting of the *sel-12* coding and 3′ untranslated region, and is expected to be stabilized in a *smg-1* background (15). pIB1Z17 was injected at a concentration of 10 μg/ml into *smg-1 unc-54* hermaphrodites. Nine independent lines containing extrachromosomal arrays were established. Four independent integrated lines were generated (using the method of C. Kari, A. Fire, and R. K. Herman, personal communication) from two of the extrachromosomal arrays. All integrated and seven of the nine extrachromosomal arrays displayed staining; all staining lines had similar expression patterns, but some lines carrying extrachromosomal arrays displayed more variability in intensity or penetrance of staining. The integrated lines all appeared to have comparable staining patterns, so we focused

on analyzing the expression pattern of the line containing the integrated array *arIs17* as a representative line.

Mixed stage populations were grown at 25°C, fixed using an acetone fixation protocol (20) and stained for β-galactosidase activity overnight at room temperature. Stained nuclei were identified based on their size, shape, and position (21, 22). Counterstaining with 4′,6-diamidino-2-phenylindole (DAPI) allowed visualization of all nuclei in the animal by fluorescence microscopy, facilitating the unambiguous identification of stained nuclei. Pictures of the staining pattern were taken at ×1000 using TMAX400 (Kodak) film.

RESULTS

A Presenilin Functional Assay. There are currently no biochemical assays for presenilin activity, so there has been no direct assay for the effects of mutations on presenilin function. The high level of similarity of SEL-12, PS1, and PS2 suggested that the ability to rescue the distinctive egg-laying defective (Egl) phenotype caused by mutations that reduce or eliminate *sel-12* activity (11) could serve as an assay for presenilin function. The *sel-12(ar131)* mutation is a C60S change (11) in a cysteine that is conserved in SEL-12, PS1, and PS2. *sel-12(ar131)* is a strong hypomorph in the genetic background used for the rescue experiments (Fig. 1). The pLEX vector (16), which places inserted cDNAs under the control of *lin-12* regulatory sequences, can direct sufficient expression of a full-length *sel-12* cDNA to rescue the *sel-12(ar131)* Egl phenotype (Fig. 1). Thus, as described below, the activity of normal and mutant human presenilins can be assessed by creating transgenic *C. elegans* lines expressing human cDNAs in the pLEX vector and assaying hermaphrodites for their egg-laying ability.

Rescue is assessed in transgenic lines that are created by the microinjection of plasmid DNA into the hermaphrodite germ line. This procedure generates extrachromosomal arrays, and there is some inherent variability in expression from different arrays, in part due to different numbers of copies of plasmid incorporated into the array (18). However, variability can be controlled for by examining multiple independent lines for each construct. Furthermore, arrays generated at the same concentration of injected DNA are likely to have comparable numbers of plasmid copies and, therefore, comparable levels of transgene expression (18). In all of the experiments described below, we have examined three independent lines for each construct and compare the results for lines generated at the same concentration of injected DNA.

Rescue of a *sel-12* Mutant by Wild-Type PS1 and PS2. We have assessed the ability of wild-type human PS1 or PS2 cDNAs to rescue the Egl defect of *sel-12(ar131)* hermaphrodites (Fig. 1). We found that the human proteins can efficiently substitute for SEL-12 in this assay, despite the vast evolutionary distance between nematodes and humans. The human proteins seem to be slightly less efficient than the *C. elegans* protein, but this small difference might in principle result from inefficient translation of human presenilin RNA due to the different codon usage between *C. elegans* and humans, so that less presenilin protein may be produced even if a comparable level of mRNA is expressed from the extrachromosomal arrays. The dramatic increase in rescuing activity when PS1 or PS2 is expressed using *lin-12* regulatory sequences, even at a relatively low concentration of injected DNA (Fig. 2), suggests that the human proteins are substituting for *C. elegans* SEL-12. An alternative interpretation is that the human protein functions in this assay by stabilizing the mutant endogenous SEL-12(ar131) protein. However, this interpretation seems less likely in view of the efficient rescue; furthermore, a corrective interaction of this sort would imply that a SEL-12 and PS1 or PS2 complex is functional, which in itself would be evidence for functional similarity of the *C. elegans* and human proteins.

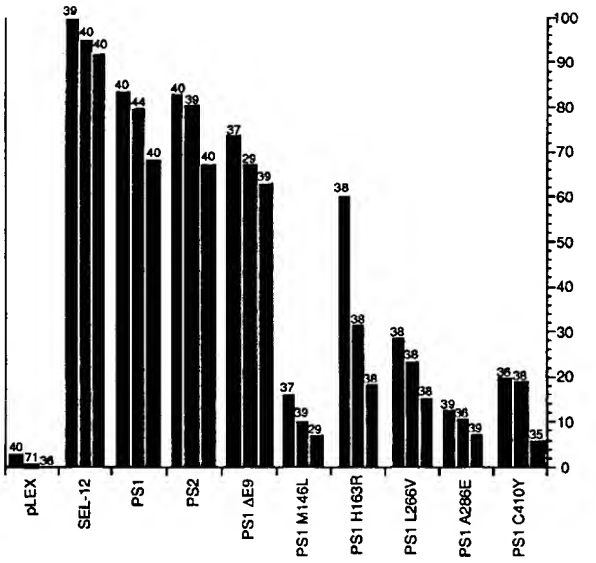


FIG. 1. Rescue of the *sel-12* Egl and abnormal vulva phenotypes by normal and mutant human presenilins. The data are shown for transgenic lines generated by injecting the construct being tested at a concentration of 20 μg/ml. Each line in the histogram represents data for an independent transgenic line; the number of hermaphrodites scored is shown above each line. The transgene is indicated on the horizontal axis. The percentage of Egl$^+$ hermaphrodites is indicated on the vertical axis. Egl$^+$ signifies robust egg-laying after 2 days; this criterion is very stringent and underestimates the degree of rescuing activity. The ability of PS1 point mutant proteins (data not shown) and the PS1ΔE9 mutant protein (see Fig. 2) to rescue *sel-12(ar131)* was further reduced when transgenic lines were generated by injecting DNA at a concentration of 2 μg/ml. Most PS1 mutations that cause Alzheimer disease affect amino acids that are identical in SEL-12. The N termini of PS1, PS2, and SEL-12 are not well conserved and are of different lengths. Therefore, for the mutations used herein, the amino acid corresponding to Met-146 in PS1 is Met-115 in SEL-12; PS1 His-163 is SEL-12 His-132; PS1 Ala-246 is SEL-12 Val-216; PS1 Leu-286 is SEL-12 Leu-255; PS1 Cys-410 is SEL-12 Cys-384. The ΔE9 mutation inhibits cleavage of PS1 (17); we note that SEL-12 is cleaved in a comparable position (24). Note that the *sel-12* cDNA used (11) has a frameshift mutation, beginning at codon 413, resulting in the substitution of 31 amino acids C-terminal to the frameshift mutation by 49 amino acids.

Activity of PS1 Point Mutants. We expressed five different human mutant PS1 proteins, each containing a single amino acid alteration that causes Alzheimer disease, and found that they displayed reduced ability to rescue *sel-12(ar131)* relative to wild-type PS1 (Fig. 1). These data suggest that the mutations that cause Alzheimer disease may reduce but not eliminate normal presenilin activity. The variable loss of extrachromosomal arrays confounds any determination of steady-state protein levels, so we do not know if the apparently lower activity of mutant presenilins results from reduced protein stability or reduced function.

Activity of PS1ΔE9. PS1 is subject to endoproteolysis *in vivo*, and the PS1ΔE9 mutant fails to be cleaved (17). We have found that the human mutant PS1ΔE9 retains a high level of activity, when arrays are formed at the injected DNA concentration of 20 μg/ml (Fig. 1). Since arrays generated at a concentration of 20 μg/ml of injected DNA are likely to contain many plasmid copies, which might mask a small difference in relative activity of PS1 and PS1ΔE9, we generated arrays at the injected DNA concentration of 2 μg/ml. At this concentration of injected DNA, the number of copies of plasmid present in the the arrays should be reduced roughly tenfold (18). At this lower concen-

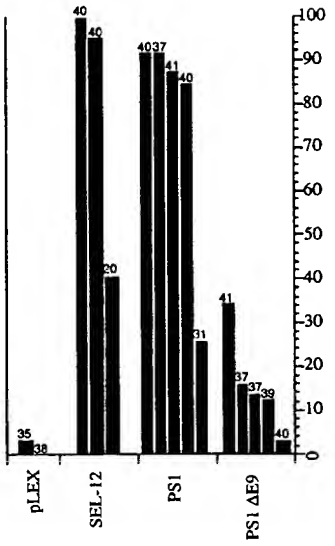


FIG. 2. Rescue of the *sel-12* Egl phenotype by PS1 and PS1ΔE9 expressed from arrays formed at a concentration of 2 μg/ml. Each line in the histogram represents data for an independent transgenic line; the number of hermaphrodites scored is shown above each line. The transgene is indicated on the horizontal axis. The percentage of Egl$^+$ hermaphrodites (see Fig. 1) is indicated on the vertical axis. At 2 μg/ml of injected DNA, expression from arrays or representation of the plasmid in the arrays may be reduced, accounting for the reduced activity of SEL-12 (transgenic line 3) and PS1 (transgenic line 5) compared with arrays generated at 20 μg/ml (Fig. 1).

tration, PS1ΔE9 has reduced ability to rescue *sel-12(ar131)* as compared with wild-type PS1 (Fig. 2), suggesting that PS1ΔE9, like the PS1 missense mutations, has reduced activity.

Examination of PS1 Mutant Transgenes in a *sel-12*(+) Background. In an attempt to reveal gain-of-function activity, we assayed the ability of transgenes encoding mutant presenilins to cause phenotypes in a *sel-12*(+) background. Antimorphic activity may in principle have caused transgenic hermaphrodites to display the Egl defect associated with reduced *sel-12* activity or defects associated with reduced *lin-12* activity; hypermorphic activity may in principle have caused egg-laying or vulval defects associated with elevated *lin-12* activity (see ref. 11). However, we saw no evidence for gain-of-function activity by these criteria (data not shown). Since intrinsic limitations of the pLEX expression system may have masked moderate changes in *sel-12* or *lin-12* activity, a definitive assessment of the gain-of-function activity of mutant presenilins in *C. elegans* will not be possible until other expression systems or strategies are developed.

***sel-12* Is Widely Expressed in Neural and Nonneural Cells.** We have examined the expression pattern of transgenic lines carrying a *sel-12::lacZ* reporter gene. Using this reporter gene, we have found that *sel-12*, like human presenilins (4, 5), is widely expressed in neural as well as nonneural cells (Fig. 3). Staining was seen in most cell types at all developmental stages from embryo to adult, with the notable exception of the intestine.

DISCUSSION

Sequence analysis revealed that SEL-12 is similar to human presenilins (11). Herein, we have provided experimental evidence that SEL-12 is a *bona fide* presenilin, since it may be functionally replaced by either of the two human presenilins. We have also shown that *sel-12* is widely expressed in most neural and nonneural tissues of developing animals and adults, as has been observed for human presenilins (4, 5). Furthermore, SEL-12 and PS1 also appear to have similar membrane topology (23, 24). These striking parallels between *C. elegans* and human presenilins suggest that studies of SEL-12 in *C. elegans* will bear directly on fundamental issues of presenilin structure and function. In the absence of any description of proteins similar to presenilins in single-celled organisms, including *Saccharomyces cerevisiae*, it appears that *C. elegans* is the simplest practical system for studying issues relevant to the biology of presenilins *in vivo*.

Since PS1 and PS2 appear to be similar in their ability to substitute for SEL-12, they may also have overlapping func-

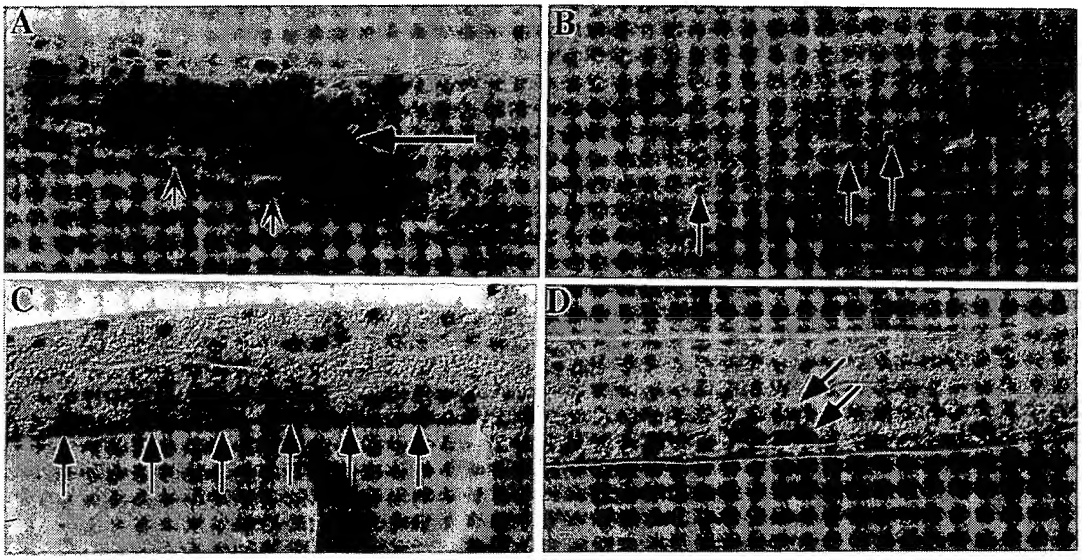


FIG. 3. Transgenic hermaphrodites expressing a *sel-12::lacZ* transgene. Expression is seen in neural and nonneural cells. (*A*) Adult. Large arrow indicates nerve ring; smaller arrows indicate muscle nuclei. (*B*) Adult. Arrows indicate ventral cord nuclei. (*C*) L3 larva. Arrows indicate nuclei of the vulval precursor cells P3.p–P8.p. (*D*). L2 larva. Arrows indicate the nuclei of the somatic gonadal cells Z1.ppp and Z4.aaa. *sel-12* activity has been shown to influence the fates of P3.p–P8.p and Z1.ppp and Z4.aaa in sensitized genetic backgrounds (11). Compromised neural function associated with reduced activity has not yet been seen in the nerve ring or ventral cord, possibly because an appropriate sensitized genetic background has not been examined. Complete genotype: *smg-1(r861) unc-54(r293); arIs17* [pRF4, pIB1Z17].

tions in mammals. As a consequence, studies of normal and mutant PS1 proteins should be directly applicable to PS2 and vice versa. Furthermore, since PS1 and PS2 have broad and overlapping expression patterns (4, 5), the phenotype of mutants homozygous for null alleles of individual mouse presenilin genes may be less severe than the phenotype of double mutants, since there may be functional redundancy where the expression patterns overlap.

The rescue experiments also provide an indication that two regions of the presenilins are not essential for normal function. (*i*) A SEL-12 protein lacking the last 31 amino acids is highly functional (see Fig. 1), suggesting that the C terminus is dispensable for SEL-12 function. (*ii*) The PS1ΔE9 protein, which lacks 30 amino acids and fails to be proteolytically cleaved (17), retains considerable activity, suggesting that neither the deleted region nor cleavage is a prerequisite for presenilin activity in *C. elegans*. We note that our rescue experiments do not address the possibility that the various mutations we tested have gain-of-function activity. Although the nature of the hypothetical gain-of-function activity of mutant presenilins is not clear in humans, the mutant presenilins appear to increase the extracellular concentration of β-amyloid-(1–42 or –43) (25, 26) and hence may cause Alzheimer disease by fostering β-amyloid deposition.

By expressing human genes in *C. elegans*, we have obtained evidence that six different presenilin mutations that cause early-onset Alzheimer disease lower normal presenilin activity. Hypomorphic characteristics were manifested as reduced ability to rescue a *C. elegans* mutant defective in *sel-12* presenilin function. In the absence of any other assays for normal presenilin function, this information may be useful in considering the pathogenesis of Alzheimer disease, and the development of mammalian models for the disease. It is possible that reduced presenilin activity may contribute to the development of Alzheimer disease, either directly or in conjunction with an as yet unknown gain-of-function activity associated with mutant presenilins.

We thank Richard Ruiz for plasmid preparations. This work was supported by National Institutes of Health Grants AG0514 and NS20471 and grants from the Adler Foundation, Alzheimer's Association, and the Develbiss Fund (to S.S.S.). D.L. was a Postdoctoral Associate of the Howard Hughes Medical Institute. T.G.D. is a predoctoral fellow of the National Science Foundation, and I.G. is an Associate Investigator of the Howard Hughes Medical Institute.

1. Schellenberg, G. D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8552–8559.
2. Clark, R. F., Cruts, M., Korenblat, K. M., He, C., Talbot, C., Van Broeckhaven, C. & Goate, A. M. (1995) *Human Mol. Genet.* **4,** 1347–1354.
3. Levy-Lahad, E., Wysman, E. M., Nemens, E., Anderson, L., Goddard, K. A. B., Weber, J. L., Bird, T. D. & Schellenberg, G. D. (1995) *Science* **269,** 973–977.
4. Rogaev, E. I., Sherrington, R., Rogaeva, E. A., Levesque, G., Ikeda, M., *et al. (1995) Nature (London)* **376,** 775–778.
5. Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., *et al.* (1995) *Nature (London)* **375,** 754–760.
6. Kovacs, D. M., Fausett, H. J., Page, K. J., Kim, T.-W., Moir, R. D., Merriam, D. E., Hollister, R. D., Hallmark, O. G., Mancini, R., Felsenstein, K. M., Hyman, B. T., Tanzi, R. E. & Wasco, W. (1996) *Nat. Med.* **2,** 224–229.
7. Muller, H. J. (1932) *Proc. Int. Congr. Genet.* **6,** 213–252.
8. Timmons, L., Xu, J., Hersperger, G., Deng, X. F. & Shearn, A. (1995) *J. Biol. Chem.* **270,** 23021–23030.
9. Wiedau-Pazos, Goto, J. J., Rabizadeh, S., Gralla, E. B., Roe, J. A., Lee, M. K., Valentine, J. S. & Bredesen, D. E. (1996) *Science* **271,** 515–518.
10. Borchelt, D. R., Lee, M. K., Slunt, H. S., Guarnieri, M., Xu, Z. S., Wong, P. C., Brown, R. H., Jr., Price, D. L., Sisodia, S. S. & Cleveland, D. W. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8292–8296.
11. Levitan, D. & Greenwald, I. (1995) *Nature (London)* **377,** 351–354.
12. Sundaram, M. & Greenwald, I. (1993a) *Genetics* **135,** 755–763.
13. Brenner, S. (1974) *Genetics* **77,** 71–94.
14. Hodgkin, J., Papp, A., Pulak, R., Ambros, V. & Anderson, P. (1989) *Genetics* **123,** 301–313.
15. Pulak, R. & Anderson, P. (1993) *Genes Dev.* **7,** 1885–1897.
16. Struhl, G., Fitzgerald, K. & Greenwald, I. (1993) *Cell* **74,** 331–345.
17. Thinakaran, G., Borchelt, D. R., Lee, M. K., Slunt, H. H., Spitzer, L., Kim, G., Ratovitsky, T., Davenport, F., Nordstedt, C., Seeger, M., Hardy, J., Levey, A. I., Gandy, S. E., Jenkins, N. A., Copeland, N. G., Price, D. L. & Sisodia, S. S. (1996) *Neuron* **17,** 181–190.
18. Mello, C. C., Kramer, J. M., Stinchcomb, D. T. & Ambros, V. A. (1991) *EMBO J.* **10,** 3959–3970.
19. Fire, A., Harrison, S. W. & Dixon, D. (1990) *Gene* **93,** 189–198.
20. Fire, A. (1993) *Genet. Anal. Tech. Appl.* **9,** 151–158.
21. Sulston, J. & Horvitz, H. R. (1977) *Dev. Biol.* **56,** 110–156.
22. Kimble, J. & Hirsh, D. (1979) *Dev. Biol.* **81,** 208–221.
23. Doan, A., Thinkaran, G., Borchelt, D. R., Slunt, H. H., Ratovitsky, T., *et al.* (1996) *Neuron*, in press.
24. Li, X. & Greenwald, I. (1996) *Neuron*, in press.
25. Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., *et al.* (1996) *Nat. Med.* **2,** 864–870.
26. Borchelt, D., Thinkaran, G., Eckman, C. B., Lee, M. K., Davenport, F., *et al.* (1996) *Neuron*, in press.